A
Synopsis
on

‘‘Isolation and characterization of Indigenous *Bacillus* species and their Biocontrol of *Fusarium* wilt in Tomato Plants’’

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SYNOPSIS

INTRODUCTION:

Tomato (*Solanum lycopersicum*) stands as one of the most economically significant and nutritionally vital crops world-wide belongs to the Solanaceae family, contributing to global food security and dietary diversity. Botanically classified as a berry, it can be grown both in the field and under greenhouse conditions (Saudi J. Biol. Sci.,2021). Numerous varieties of the tomato plant are widely grown in temperate climates across the world, with greenhouses allowing for the production of tomatoes throughout all seasons of the year and typically grow to 1-3 meters in height. Tomatoes are a rich source of essential nutrients such as vitamins A and C, potassium, and lycopene-a powerful antioxidant linked to various health benefits. Their culinary versatility ranges from raw consumption in salads to being transformed into sauces, soups, juices, and sundry processed products.

The agricultural landscape of Samastipur faces an imminent threat from *Fusarium* wilt, a debilitating disease affecting tomato plants and imposing substantial economic burdens on farmers. Among the fungal diseases of tomato, *Fusarium* wilt is one of the most serious ones throughout the world. India ranks second in the production of tomato crop. The state Andhra Pradesh is the largest producer of tomato in India. The other major producer of tomato in India are Bihar, Uttar Pradesh, Madhya Pradesh, West Bengal and Orrisa etc., (Abhirath, Bhaswati Chakraborty et al.,2022).

Fusarium oxysporum, the causal agent of this soil-borne pathogen, continues to challenge traditional methods of disease management, often reliant on chemical pesticides with inherent environmental and ecological drawbacks. As the quest for sustainable agricultural practices intensifies, there is a compelling need for innovative, eco-friendly solutions to mitigate the impact of *Fusarium* wilt on tomato crops. Agricultural sustainability faces challenges with conventional disease management practices, particularly in the context of *Fusarium* wilt affecting tomato plants.

This research aims to bridge existing knowledge gaps by specifically focusing on indigenous *Bacillus* species in Samastipur for *Fusarium* wilt biocontrol. This research aims to isolate and characterize indigenous *Bacillus* species for their bio-control potential against *Fusarium* wilt in tomato plants in Samastipur.

REVIEW OF LITERATURE:

In India and around the world, several works have been undertaken to control various diseases of tomato by using antagonistic microorganisms. Previous studies highlight the effectiveness of *Bacillus* species as bio-control agents against various plant pathogens (Smith *et al*., 2019; Khan *et al.,* 2020). The adaptability and specificity of indigenous *Bacillus* strains make them promising candidates for sustainable disease management (Dey *et al*., 2018). However, the specific contributions of native *Bacillus* species from the Samastipur region remain largely unexplored.

Bacillus Species in Bio-control: Recent studies (Xu *et al*., 2023; Sharma and Patel, 2022) underscore the potential of Bacillus species as effective bio-control agents against various plant pathogens. Their ability to suppress diseases through mechanisms like antibiosis, competition, and induced systemic resistance highlights their significance in sustainable agriculture.

Molecular Characterization Techniques: Advancements in molecular techniques (Li *et al*., 2024) offer sophisticated tools for the characterization of *Bacillus* species. Genomic transcriptomic, and metabolomic analyses provide insights into the genetic diversity and functional capabilities of indigenous *Bacillus* strains.

Bio-control Mechanisms: Understanding the bio-control mechanisms of *Bacillus* against *Fusarium* wilt is crucial. Recent work by Chen *et al.* (2023) illuminates the molecular and physiological interactions between *Bacillus* species and *Fusarium oxysporum*, shedding light on potential targets for bio-control strategies.

OBJECTIVES:

1. To observe symptoms of the disease, sample it, isolate plant pathogen, and after culturing and subculturing, identify the pathogen.
2. To collect soil samples from the local agro-ecosystem in Samastipur for isolation of Indigenous *Bacillus* species to evaluate the potential control effects on *Fusarium*.
3. To evaluate the antagonistic activity.
4. To perform Biochemical, Morphological characterization of isolated *Bacillus* species.
5. To determine the impact of Bacillus-based bio-control on the overall health and yield of tomato plants.
6. To monitor the parameters such as disease incidence, plant growth, and fruit quality
7. To assess the effectiveness and sustainability of the bio-control strategy.

PLAN OF WORK:

(a) Collection and isolation of pathogens from the infected part of Tomato stem, leaves and fruits from several locations in Samastipur and brought to the laboratory immediately for pathogen isolation.

(b) Testing of pathogenicity of the organisms involved in disease or syndrome.

(c) Preparation of pure culture of pathogen and antagonistic microorganism may be involved. Bacillus species- *Bacillus subtilis, Bacillus amyloliquefaciens, B. pumilus,*. Bacteria with inhibitory characteristics can be selected and screened to evaluation of Antagonistic Activity of Bacillus isolates.

(d) Investigation of the impact of *Bacillus*-based bio-control on the overall health and yield of tomato plants. Parameters such as disease incidence, plant growth, and fruit quality will be monitored to assess the effectiveness and sustainability of the bio-control strategy.

(e) Field Trials: Following the footsteps of Singh *et al.* (2022), selected *Bacillus* strains will undergo rigorous field trials to evaluate their performance under natural conditions in Samastipur, providing insights into their practical applicability.

METHODOLOGY:

(a) Sampling of Plant Materials: To Select plants showing typical symptoms of *Fusarium* wilt, such as wilting, yellowing, and vascular discoloration. Affected/Necrotic areas (root, stem, leaves, and fruits) are cut into small pieces and will be surface sterilized using 70% (v/v) ethanol (5 min), 2.5% (v/v) NaOCl (5min). 0.1% $HgCl_2$ (about 30sec.) followed by washing three times with sterile distilled water etc.

(b) Isolation of *Fusarium oxysporum*: Surface sterilize symptomatic plant tissues to eliminate external contaminants. Homogenize plant tissues using a sterile mortar and pestle to release fungal spores. Plate homogenized tissues onto selective media such as Potato Dextrose Agar (PDA) supplemented with appropriate antibiotics to inhibit bacterial contamination. Incubate plates at the optimum temperature for *Fusarium oxysporum* growth (typically 25-30°C).

***(c)* Identification and Culture of Pathogen:** The fungal colonies for typical *Fusarium* characteristics, including colony color, texture, and sporulation will be observed.

(d) Microscopic Analysis: Perform microscopic examination of fungal hyphae and spores to confirm *Fusarium* morphology.

(e) Isolation of *Bacillus* Species: Use selective media containing antibiotics to suppress non-Bacillus microorganisms. Employ serial dilution and spread plate methods for isolation. Incubate plates at optimal temperature (e.g., 30-37°C) for Bacillus growth. Perform Gram staining and observe under a microscope. Subsequently, conduct Biochemical tests for preliminary identification.

(f) Identification and Culture of *Bacillus* Species: The isolated bacteria are then cultured on appropriate media to encourage their growth. Nutrient Agar is a commonly used medium for Bacillus species. Bacteria may be identified using Gram Staining, Catalase activity, acidity test, and some ready-made test kit.

(g) Screening and Evaluation of Antagonistic Activity Against *Fusarium oxysporum*: Acquire a virulent strain of *Fusarium oxysporum* isolated from infected tomato plants. Perform in vitro dual culture assays to assess antagonistic activity. Measure the inhibition zone and document observations for each *Bacillus-Fusarium* interaction.

(h) Bio-Control Efficacy Assessment: Greenhouse Experiment: Inoculate tomato plants with *Fusarium oxysporum* and treat with selected *Bacillus* strains.

(i) Statistical Analysis: Use appropriate statistical tests to evaluate significant differences between treatments

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